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Dynamin Assembly Strategies and Adaptor Proteins in Mitochondrial Fission

Review

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Mitochondrial fission is mediated by a dynamin-related GTPase that assembles at constricted sites on the organelle. The mechanism of action of this GTPase in fission is related to that of classical dynamin, which severs the necks of clathrin-coated pits at the plasma membrane. The scale of these membrane remodeling events differs by an order of magnitude, however, and structural studies have revealed variations in the assembly properties of classical and mitochondrial dynamins that accommodate these differences. Despite this progress, structural and mechanistic models have not yet incorporated a growing number of adaptor proteins that are required for the membrane recruitment and function of mitochondrial dynamins. Here, we review the structure and assembly properties of the yeast and mammalian mitochondrial dynamins and discuss what is known about the activities of their adaptor proteins.

Introduction

Mitochondria are complex double-membrane organelles in eukaryotic cells. The most widely recognized role for mitochondria is to generate ATP for diverse cellular functions. However, mitochondria are indispensable for life, even in cells that do not depend on respiration, because they are required for essential processes, including the assembly of iron–sulphur clusters, the citric acid cycle, fatty acid β-oxidation, and heme and phospholipid biosynthesis. In addition, mitochondria are key regulators of developmental processes, aging and apoptosis. In many eukaryotic cells, mitochondria are tubular, branched and distributed throughout the cytoplasm. Mitochondria constantly fuse, divide and move along cytoskeletal tracks. These dynamic activities control their morphology and intracellular distribution and determine their cell-type-specific appearance.

The number and shape of mitochondria is governed by the balance of fusion and fission events [1–3]. Fusion generates the mitochondrial network, which is thought to be important for the dissipation of metabolic energy along mitochondrial tubules. Fusion of individual organelles back to the network is also proposed to slow features of aging attributed to the accumulation of mitochondrial DNA (mtDNA) mutations and reactive oxygen species (ROS) [4,5]. According to this model, the healthy mitochondrial network provides mtDNA copies to complement mtDNA mutations brought in by damaged mitochondria [6]. By contrast, fission generates smaller compartments, which are more readily transported along cytoskeletal tracks, transferred to daughter cells during division, and turned over by mitophagy [7–10].

Mitochondrial fission is mediated by self-assembling GTPases called dynamin-related proteins (DRPs). The prototype for this family, classical dynamin, interacts directly with the lipid bilayer at the necks of clathrin-coated pits at the plasma membrane to sever and release coated vesicles [11–14]. By contrast, the mitochondrial DRPs bind to adaptor proteins associated with the outer mitochondrial membrane (Figure 1A). Assembly is favored at sites where a tubule from the endoplasmic reticulum crosses and induces a constriction of the mitochondrial tubule [15]. Recruitment of the mitochondrial dynamin to the adaptor at these constrictions is followed by assembly of this DRP into multimeric structures (Figure 1A) [16–19]. The assembly process also drives the reorganization and multimerization of the adaptors to form active fission complexes [20–25]. Loss of function of the mitochondrial dynamin or its adaptor proteins causes fission defects, in which unopposed fusion of mitochondrial tubules generates an interconnected, 'net'-like and collapsed mitochondrial morphology [18,19,26,27].

Although mitochondrial dynamins are conserved from yeast to human, their membrane adaptors appear to have evolved independently and are not related by primary amino acid sequence, predicted secondary structure, or domain composition (Figure 1B). Moreover, mammals express multiple adaptors that regulate recruitment and assembly of a single dynamin. In this review, we discuss how this increased complexity impacts standing models for mitochondrial dynamin assembly and function. We also discuss how a conserved mitochondrial dynamin could co-evolve with diverse adaptors while retaining its basic fission function.

The Mitochondrial Fission Dynamin

Yeast and mammals express one mitochondrial fission dynamin called Dnm1/Drp1 (yeast/mammals). Though not discussed in this review, the same dynamins carry out peroxisome division in these organisms [28,29]. Like classical dynamin, the linear Dnm1/Drp1 sequences contain conserved GTPase, middle, and GTPase effector (GED) domains (Figure 2A). However, Dnm1/Drp1 lacks the pleckstrin homology (PH) and carboxy-terminal proline-rich domains (PRD) that are found in dynamin. In place of the PH domain, Dnm1/Drp1 contains a unique sequence called insert B (InsB) [30]. *In vivo*, Dnm1/Drp1 assembles into structures that can completely encircle and divide mitochondrial tubules [19,31,32]. However, the steady-state localization of this mitochondrial GTPase differs in yeast and mammalian cells. Under standard laboratory growth conditions, yeast Dnm1 is found in many well-spaced complexes that appear as puncta on mitochondrial tubules [16,18]. By contrast, Drp1 is largely cytoplasmic in a variety of mammalian cells grown in culture and assembles onto mitochondria in response to specific cellular cues [27,33]. In both yeast and mammals, loss of Dnm1/Drp1 blocks the formation of fission complexes, despite normal expression and mitochondrial localization of other fission proteins [20,25,34].

Surprisingly, both yeast cells and mouse embryo fibroblasts (MEFs) lacking Dnm1/Drp1 survive and are able to partition parts of the interconnected mitochondrial network into daughter cells during mitotic division [35,36]. However, segregation of this structure during meiosis is apparently more challenging, and yeast lacking Dnm1 often produce

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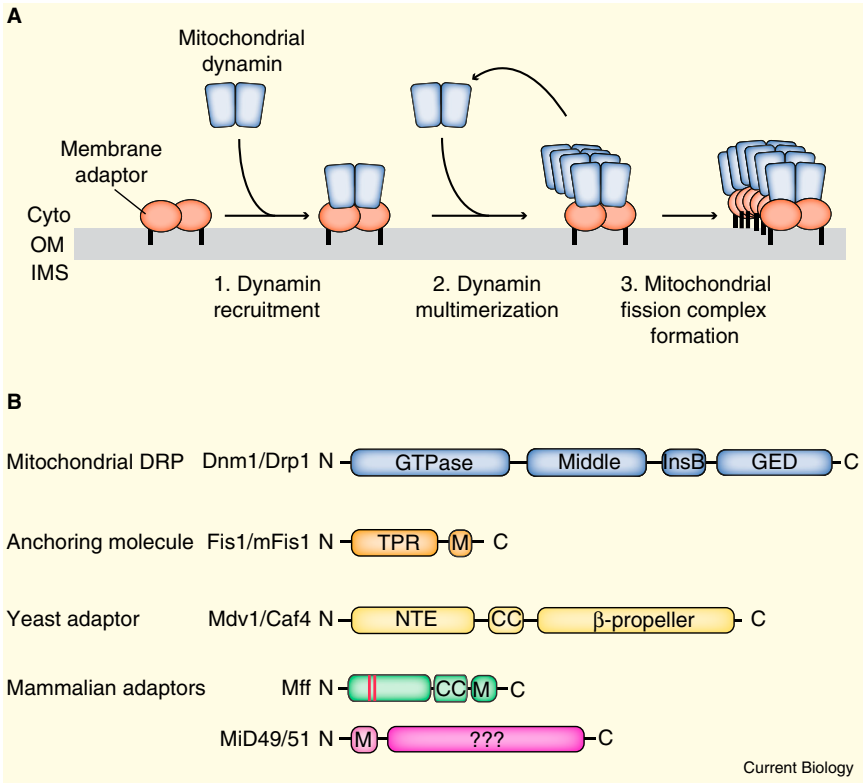

Figure 1. The mitochondrial fission machinery.

(A) Stepwise model of general mitochondrial fission complex assembly. Cyto, cytoplasm; OM, outer mitochondrial membrane; IMS, intermembrane space. (B) Yeast and mammalian mitochondrial fission proteins. Dnm1, dynamin-related protein 1; Drp1, dynamin-related protein 1; InsB, insert B; GED, GTPase effector domain; Fis1, fission 1; TPR, tetratricopeptide repeat; M, transmembrane domain; Mdv1, mitochondrial division 1; Caf4, CCR4-associated factor 4; NTE, amino-terminal extension; CC, coiled-coil; Mff, mitochondrial fission factor (short repeats are indicated in red on Mff); MiD, mitochondrial dynamics. MiD51 is also called MIEF1, mitochondrial elongation factor 1 [68]. '???' denotes no recognizable motifs or domains.

inviable meiotic progeny (spores) that lack mitochondria [37]. Drp1-mediated mitochondrial fission is essential during development of multicellular eukaryotes. In worms [19] and mice [35,36], disruption of Drp1 causes embryonic lethality. In addition, a human Drp1 mutation was reported in an infant who died 37 days after birth with microcephaly, abnormal brain development, optic atrophy, and lactic acidemia [38]. Altered Drp1 abundance and/or function has also been linked to a variety of diseases, including Parkinson's disease [39], Alzheimer's disease [40], and Huntington's disease [41,42].

Classical Dynamin Structure, Assembly and Mechanism of Action

Our understanding of dynamin GTPases was significantly advanced by structural studies of dynamin [43–47] and another member of this family, MxA [48,49]. Owing to their conserved sequence, the mitochondrial dynamins are predicted to adopt a four-domain architecture similar to dynamin, which includes a GTPase domain, a bundle signaling element (BSE), a stalk, and a PH/InsB domain (Figure 2A). This architecture is similar to that depicted in Figure 2B for dynamin lacking its PRD domain (DynΔPRD).

Structural studies and *in vitro* assays using purified dynamin defined at least three distinct protein conformations depending on its nucleotide association status. The conformational changes are induced by GTP binding and GTP hydrolysis. Conformation 1 represents assembly-deficient dynamin in solution in the absence of nucleotide [46,47]. This conformation is believed to exist only transiently *in vivo* because of dynamin's low micromolar affinity for guanine nucleotide, and the high intracellular GTP concentration (1 mM) [50]. Conformation 2 represents GTP-bound dynamin oligomerized on a membrane surface. This structure was solved by cryo-electron microscopy (cryo-EM) reconstruction using purified dynamin in the presence of the non-hydrolyzable GTP analog GMPPCP [43]. Although dynamin can assemble into spirals in the absence of nucleotide *in vitro* [51], it is recruited to the membrane in the GTP-bound form *in vivo* [52–54]. Thus, binding of GTP likely induces the switch from conformation 1 to conformation 2, which favors membrane association. Conformation 3 represents oligomerized dynamin during the GTP hydrolysis transition state. This conformation was derived from the crystal structure of an artificial dynamin GTPase–GED fusion (GG) in the presence of the transition state analog GDP·AlF_4^- [44]. These nucleotide-driven conformational changes are critical for dynamin-mediated membrane constriction and fission, and are frequently used to model the behavior of other DRPs, including the mitochondrial dynamins.

The crystal structure of the stalk of the DRP MxA revealed that DRPs self-assemble via three interfaces in the stalk region (Figure 2C) [49]: interface 1 is at the top of the stalk, below the BSE; interface 2 is located in the center of the stalk; and interface 3 is at the bottom of the stalk, right above the PH/InsB domain. The stalk dimerizes in a cross-like manner at interface 2, forming the dimeric dynamin building block (Figure 2B). Interactions at interfaces 1 and 3 are established only during assembly of dynamin dimers into strands composed of neighboring dimers. These strands wrap around lipid tubes forming rings and spirals. In the structure of the MxA stalk region, the stalk interactions result in a one-ring assembly [48]. In the DynΔPRD nucleotide-free crystal structure, interface 1 is smaller [47] or not formed [46]. Instead, the dynamin stalks are tilted relative to the stalk axis. However, because this surface is hydrophobic and its residues are highly conserved, it is likely to play a similar role in dynamin and MxA assembly. It is possible that interface 1 is only fully formed during dynamin assembly and induces a pitch that favors the spiral complexes in dynamin instead of the ring-like structure observed for MxA. Indeed, the direct interactions at all stalk interfaces fit into the cryo-EM reconstruction of GMPPCP-bound DynΔPRD on liposomes (conformation 2). This structure reflects the

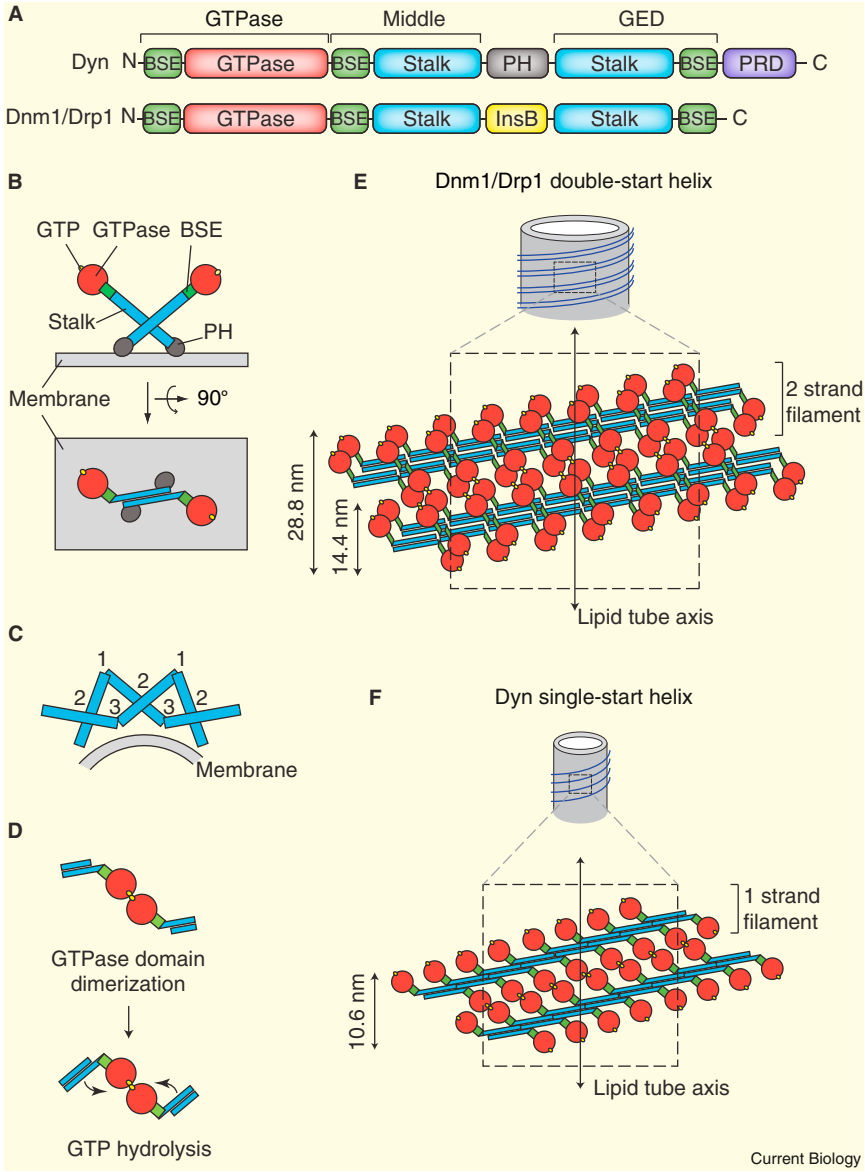

Figure 2. Mitochondrial dynamin structure and assembly.

(A) Linear domain organization of classical dynamin (Dyn) and mitochondrial dynamin (Dnm1/Drp1). (B) End-on and top-down views of Dyn *in vitro* assembly on the membrane tube. (C) Interfaces in the stalk region mediate dynamin dimerization (interface 2) and polymerization (interface 1 and 3). (D) The dimerization of GTPase domains (in the GTP-bound state) between adjacent strands of the dynamin spiral stimulates GTPase hydrolysis, resulting in conformational changes that constrict and sever the membrane. (E,F) Schematic representation of double-start Dnm1/Drp1 helix (E) and single-start Dyn helix (F) assembled around lipid tubes. The bound nucleotide in D–F is indicated in yellow. GED, GTPase effector domain; PRD, proline-rich domain; BSE, bundle signaling element; PH, pleckstrin homology; InsB, insert B. Regions that belong to the same structural domain are shown in the same color.

conformational changes induced by GTP binding and is consistent with the observation that dynamin is recruited to the membrane in the GTP-bound form [52–54].

In a dynamin spiral, the dynamin molecules interact via the three stalk interfaces, forming the 'spiral backbone' of a strand (Figure 2E). GTPase domains project from the backbone in opposite directions [46,47]. The GTPase domains of molecules in two adjacent rungs of the spiral dimerize via a fourth interface identified in the crystal structure of the dynamin GG construct (Figure 2D) [44]. GG is a monomer in the absence of nucleotides but dimerizes in the presence of GTP or GDP·AlF_4^-, suggesting that dimerization of the GTPase domain occurs prior to and during GTP hydrolysis. Dimerization of the GTPase domain in dynamin assemblies organizes the components of the GTPase active site, leading to a dramatic stimulation of GTPase activity (>100-fold) [50,55,56]. This GTP hydrolysis induces a hinge-like conformational change in the BSE (Figure 2D) [44,45]. Although the details have not been resolved, propagation of this conformational change along the dynamin assembly could lead to constriction and fission of an underlying lipid tube. Consistent with this model, previous studies have demonstrated that dynamin alone will sever lipid tubes in the presence of hydrolyzable GTP [57–60]. These conformational changes are also predicted to disrupt the interaction at stalk interface 1 and lead to dynamin disassembly [59,60]. Forces introduced during the disassembly process could also contribute to membrane scission.

Mitochondrial Dynamin Structure, Membrane Assembly and G Domain Interaction

Structural studies have identified fundamental differences between classical dynamin and Dnm1/Drp1 in assembly architecture. These differences are consistent with the *in vivo* specialization of Dnm1/Drp1 for mitochondrial membrane constriction.

In vitro, both Dnm1 and Drp1 exhibit assembly-stimulated GTP hydrolysis, though the extent of the stimulation (~10–15-fold) is much lower than that of dynamin (>100-fold) [11,56,61–63]. Both mitochondrial dynamins also assemble into spirals that deform liposomes into tubes and constrict these tubes upon GTP hydrolysis [62–65]. However, cryo-EM reconstructions reveal that Dnm1 spirals differ from dynamin spirals in diameter, architecture, and constriction capacity [65]. The diameter of constricted mitochondrial tubules is ~110 nm [64], much larger than the size of endocytic vesicle necks at ~25–30 nm [66]. As a consequence, mitochondrial fission requires a much more substantial constriction to mediate membrane scission. The assemblies formed by Dnm1 on lipids *in vitro* appear to fit these requirements [65]. First, the average outer/inner diameter of Dnm1 spirals assembled on lipid tubes in the absence of nucleotide is ~129/80 nm, again, much larger than that of dynamin spirals

(50/25 nm). Second, in the presence of GTP, the inner diameter of Dnm1 spirals constricts ~50 nm compared to ~10 nm for dynamin [43,65]. Whether the internal diameter of these constricted Dnm1 spirals is sufficient to achieve fission of both the inner and outer mitochondrial membranes remains unclear. In fact, Dnm1 and Drp1 have only been shown to constrict, not sever, lipid tubes *in vitro* [62–65].

The spirals formed by Dnm1 and dynamin differ in another important respect. When assembled on lipid tubes, the yeast mitochondrial dynamin Dnm1 forms a two-start helix with a helical pitch of 28.8 nm (Figure 2E) [65]. The axial spacing between the two starts of the Dnm1 helix is 14.4 nm. By contrast, dynamin forms a single-start helix with a helical pitch of 10.6 nm (Figure 2F) [65]. An assembly model that explains this difference was recently proposed based on the crystal structure of the mitochondrial dynamin Drp1 (also called DNM1L) [62]. In this case, the crystallized protein included the GTPase, BSE, and stalk domains but lacked the InsB domain. Based on the structure in the absence of nucleotide and on biochemical experiments, Drp1 monomers are predicted to assemble via interface 2 to form dimers and via interfaces 1 and 3 to form strands (Figure 2C). In addition, the Drp1 structure also revealed a novel assembly surface, interface 4, which is not found in dynamin or MxA. Interactions at interface 4 appear to mediate assembly between the stalks of two neighboring Drp1 strands. Thus, assembly via interface 4 would generate a broader filament composed of two adjacent Drp1 strands (Figure 2E) [62]. This model accounts for the two-start helix, larger pitch and broader strand size observed for the mitochondrial dynamin Dnm1 in cryo-EM reconstruction studies [65]. This model also proposes that G domains dimerize across neighboring two-strand filaments (Figure 2E) [62]. Presumably, GTP hydrolysis and conformational changes at these sites lead to membrane constriction and fission in a manner similar to that proposed for adjacent rungs of dynamin spirals. It is unclear whether additional G domain interactions occur within the two-strand Drp1 filament, and if so, how GTP hydrolysis at these sites would affect movements in the proposed Drp1 assembly.

Additional X-ray and cryo-EM structures of Dnm1 and Drp1 bound to GTP or GDP•AlF_4^- will help to clarify similarities and differences in the architectures and conformational changes of classical and mitochondrial dynamins. Even without this information, it is clear that members of the DRP family have evolved unique strategies to achieve optimal complex architecture and activity for their functions at different cellular membranes.

Fission Adaptor Proteins Recruit Mitochondrial Dynamins to the Membrane

Although dynamin interacts directly with the lipid bilayer via its PH domain, Dnm1 does not. Instead, in the cryo-EM reconstruction there is a gap of 3–4 nm between the membrane and the inner edge of the Dnm1 assembly [65]. *In vivo*, this gap is most likely occupied by the membrane-associated fission adaptor proteins. Thus far, two yeast (Mdv1 and Caf4) and three mammalian (Mff, MiD49 and MiD51) adaptors have been identified [20,21,24,25,67,68]. All of these proteins interact directly with their respective mitochondrial dynamin and form a physical bridge between the dynamin and the outer mitochondrial membrane. More importantly, the adaptors appear to co-assemble with the mitochondrial dynamin to form active fission complexes [22–25]. Both yeast and mammals also express a conserved membrane anchor called Fis1. However, the function of Fis1 is not fully understood, since it is strictly required for membrane recruitment of the yeast adaptors Mdv1 and Caf4, but not the characterized mammalian adaptors [34,67,69]. Fis1 is also dispensable for fission in worms [70]. The yeast Mdv1 adaptor contains an amino-terminal extension (NTE), a coiled-coil (CC) domain, and eight WD repeats predicted to form a β-propeller (Figure 1B). Mdv1's NTE domain interacts directly with Fis1 and the β-propeller domain binds directly to Dnm1 [69]. Mdv1 is required to recruit Dnm1 to the membrane and coassembles with Dnm1 into fission complexes, where it remains throughout mitochondrial division [23]. Yeast also express a second adaptor, Caf4, which is an Mdv1 paralog (Figure 1B) [67]. Although Caf4 is capable of facilitating fission as the sole adaptor and is found in fission complexes together with Mdv1, it is not essential for fission when Mdv1 is present [71].

Mammals lack an Mdv1/Caf4 ortholog and instead express three different Drp1 adaptors — Mff, MiD49 and its paralog MiD51 (also called MIEF1) — each of which is anchored in the mitochondrial outer membrane (Figure 1B) [24,25,68]. The amino-terminal cytoplasmic domain of Mff contains two short repeats and a central coiled-coil and bears no resemblance to yeast Fis1 or Mdv1/Caf4. The carboxy-terminal cytoplasmic domains of MiD49 and MiD51 contain no defining structural features and are also unrelated to Mff, Fis1 and Mdv1/Caf4.

How and when these different mammalian adaptors function is still being explored. A recent study established that Mff is required for Drp1 recruitment to the mitochondrial surface and that this adaptor colocalizes with Drp1 complexes on mitochondrial membranes [72]. Mff overexpression also increases the amount of Drp1 on mitochondria and mitochondrial fission. Although MiD49 and MiD51 can recruit Drp1 to mitochondria [25], whether they act as positive or negative regulators of fission is less clear. Supporting a role in fission, individual or simultaneous depletion of MiD49 and MiD51 causes mitochondrial elongation [25,73]. Arguing against a role in fission, overexpression of either MiD49 or MiD51 causes mitochondrial elongation rather than increased fission [25,68]. The latter result may be explained by the recent finding that overexpression of MiD49 or MiD51 enhances inhibitory phosphorylation of Drp1 on mitochondria [73]. Treatments that reverse Drp1 phosphorylation at this site reduce mitochondrial elongation. Thus, an alternative hypothesis is that Drp1 recruited to mitochondria by overexpressed MiD49 or MiD51 is maintained in an inactive state [73]. If correct, this alternative hypothesis could explain results from another group, suggesting that MiD51 negatively regulates mitochondrial fission [68].

The existence of multiple, simultaneously expressed adaptors complicates experiments in mammalian cells aimed at disentangling their relative contributions to Drp1 recruitment and fission. Moreover, few studies to date address post-Drp1 recruitment roles of adaptors, including membrane scission. The minimal combination of adaptors and Drp1 required for fission complex formation and fission was recently defined by expressing mammalian (human) proteins in yeast cells lacking the endogenous fission machinery [63]. These studies established that, when expressed individually, the Mff, MiD49 or MiD51 adaptors can recruit Drp1 to mitochondria, assemble Drp1-containing fission complexes, and complete mitochondrial fission. Tethering

the yeast Mdv1 adaptor to the mitochondrial surface was also sufficient to recruit yeast Dnm1 to mitochondria and complete fission [63]. For all adaptor–DRP combinations, time-lapse imaging confirmed that Dnm1/Drp1 assembled at sites where mitochondrial fission occurred *in vivo*. Interestingly, both yeast and human Fis1 were dispensable in this sufficiency system, consistent with the idea that Dnm1/Drp1-mediated mitochondrial fission is not the conserved function of this membrane anchor. Of course, the yeast sufficiency system is most likely missing some signaling pathways and/or modifying enzymes critical for regulation of mammalian fission proteins. Additional studies are necessary to determine: whether different combinations of mammalian adaptors work together under some circumstances to carry out Drp1-mediated fission; whether the function or availability of different mammalian adaptors varies depending on cell type; and whether different cell signaling pathways engage different mammalian adaptors.

The yeast fission adaptors Mdv1 and Caf4 depend on the membrane anchor Fis1 for targeting to the mitochondrial surface (Figure 1B). The Fis1 carboxyl terminus is embedded in the outer mitochondrial membrane, and its amino-terminal, tetratricopeptide repeat (TPR) domain faces the cytoplasm [34]. Loss of yeast Fis1 results in cytoplasmic localization of Dnm1 and Mdv1/Caf4 and impaired mitochondrial fission, consistent with its role in membrane recruitment of the fission machinery [34,67,69]. Despite its conserved structure, localization and membrane topology, strong experimental evidence for a similar function of mammalian Fis1 (mFis1) in fission has been difficult to obtain. In favor of a role in fission, inhibition of mFis1 was reported to cause mitochondrial elongation and overexpression of mFis1 was reported to cause mitochondrial fragmentation [74–78]. Arguing against a role in fission, mFis1 knockdown in HeLa cells or a conditional mFis1 knockout in a colon carcinoma cell line had no effect on mitochondrial fission or morphology [72]. A more recent analysis of mFis1 knockout MEFs concluded that mFis1 plays a measurable, but minor, role in fission [73].

A similar discrepancy exists regarding the requirement of mFis1 for Drp1 recruitment to mitochondria. Although mFis1 is reportedly precipitated in a complex with Drp1 after chemical cross-linking [78], convincing evidence that the two proteins directly interact is lacking. While several studies suggest that deletion or depletion of mFis1 does not affect Drp1 recruitment to the mitochondrial surface [72,75], a minor decrease in mitochondrial-associated Drp1 was documented in MEFs lacking mFis1 [73]. The fact that Fis1 has been conserved during evolution argues in favor of a common, and vital, cellular role for this protein. Whether mFis1-mediated mitochondrial fission is necessary in specific cell types or under selected physiological conditions is an important unsolved problem.

Structure of the Mdv1–Fis1 Yeast Mitochondrial Dynamin Adaptor Complex

Three-dimensional structures are necessary to understand how mitochondrial dynamins interact with their adaptors. Such information is currently only available for the yeast adaptor complex. Yeast Dnm1 is recruited to the mitochondrial surface by a heterotetramer containing two Mdv1 molecules and two Fis1 molecules [79,80] (Figure 3). The Mdv1 monomers dimerize via a 106 Å-long antiparallel CC domain. Two predicted β-propeller domains emerge from the opposite ends of the CC domain, and function as docking sites for cytoplasmic Dnm1 dimers. The Mdv1 NTE domains wrap around the Fis1 cytoplasmic TPR domains via a U-shaped sequence containing a helix A–loop–helix B motif [81]. Helix A interacts with the Fis1 TPR convex surface while helix B binds the concave surface. Mutations in this motif were shown to impair Mdv1–Fis1 interactions *in vivo*. The NTE–Fis1 interaction is stabilized by an additional Mdv1–Fis1 interface between the Mdv1 CC domain and the top surface of the Fis1 cytoplasmic domain [80]. Mutations in Fis1 at this site compromise recruitment of Mdv1 and reduce mitochondrial fission activity. The Mdv1 CC domain also makes an intramolecular contact with helix B. The binding of helix B to the Fis1 TPR-like concave surface is stabilized by its compression between the Fis1 amino-terminal arm and the Mdv1 CC domain. These interactions precisely orient the Mdv1 CC domain in relation to the two Fis1 molecules, while presenting the β-propeller domains for Dnm1 recruitment. The minimal oligomeric form of cytoplasmic Dnm1 has been shown to be a dimer [22], though larger Dnm1 assemblies are also observed [64]. Whether each β-propeller domain binds a single Dnm1 monomer or two monomers of the dimer is not known.

Adaptors Modulate Mitochondrial Dynamin Assembly

Adaptors not only recruit mitochondrial dynamins to the organelle surface, they also modulate dynamin assembly at the membrane. In the case of the yeast adaptor complex, the Mdv1 β-propeller domains function as physical scaffolds to orient and stabilize Dnm1 for further polymerization. The distance between the β-propeller domains in Mdv1 is determined by the antiparallel CC domain (Figure 3B). Mutagenesis studies demonstrate that both the sequence and length of this CC domain are critical for Dnm1 recruitment and assembly, and in some cases, co-assembly of Mdv1 into fission complexes [79]. Biochemical studies demonstrate that Mdv1 alone can promote Dnm1 assembly [61]. Purified Dnm1 will self-assemble *in vitro*, and this assembly stimulates its GTPase activity. The early stages of assembly are characterized by a concentration-dependent kinetic lag, which is thought to represent a nucleation step in the Dnm1 assembly pathway. *In vitro*, Mdv1 interacts specifically with the GTP-bound form of Dnm1 and promotes Dnm1 assembly by reducing this kinetic lag. Although this nucleating activity would be important after Dnm1 recruitment to initially form the fission complex, the fact that Mdv1 co-assembles with Dnm1 in complexes suggests that the adaptor may also be required after assembly for membrane constriction and/or scission.

Studies to date suggest that mammalian adaptors have a different effect on Drp1. Like other members of this family, Drp1 exhibits assembly-stimulated GTP hydrolysis, induces lipid tubulation, and in the presence of hydrolyzable GTP constricts membranes (from a diameter of ~65 nm to ~31 nm) [63]. Note that the diameter of the Drp1 spirals on lipid tubes and the degree of constriction induced by GTP hydrolysis may vary, depending on the Drp1 isoform studied and the conditions used [62]. Although Mff and MiD adaptors can individually recruit Drp1 to mitochondria and support fission complex formation *in vivo*, these adaptors do not dramatically affect Drp1's assembly-stimulated GTP hydrolysis activity *in vitro* [63]. Instead, MiD49 coassembles with Drp1, forming a heteropolymer with a much narrower diameter (~15 nm) than Drp1 alone [63]. The diameter of this

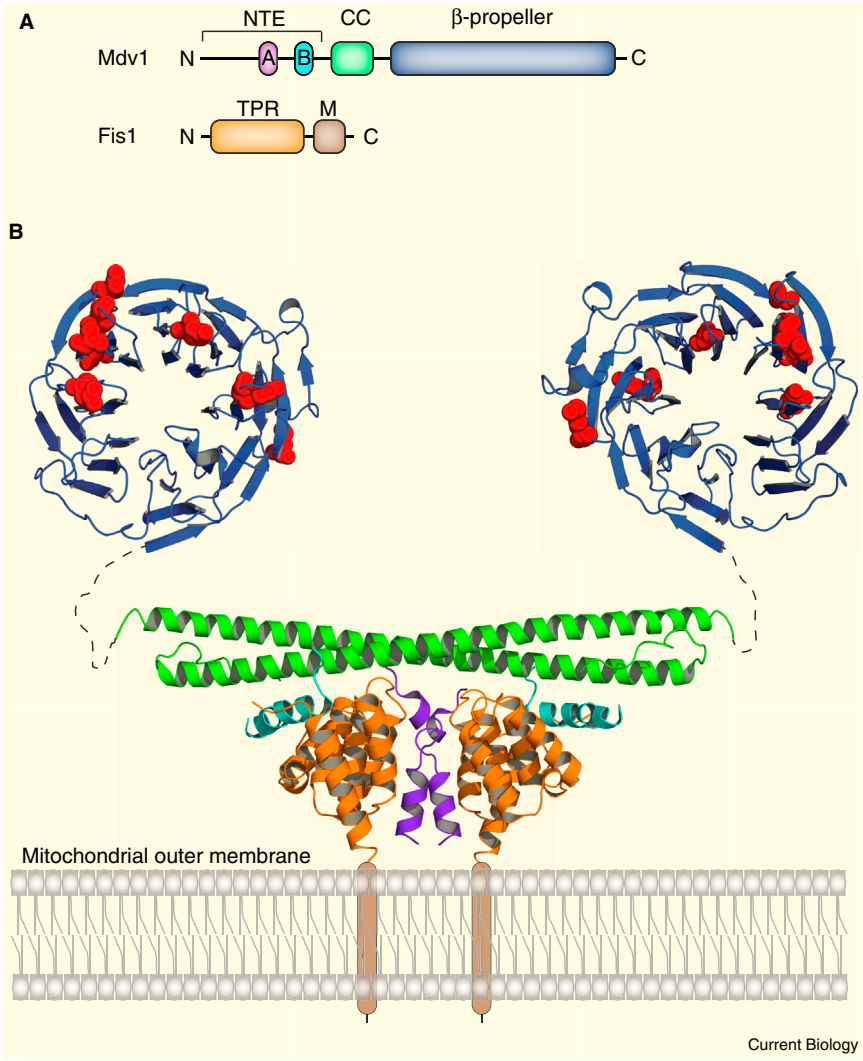

Figure 3. Molecular architecture of the yeast mitochondrial dynamin adaptor complex.

A structural model of the Mdv1 dimer (CC, green; helix A, purple; helix B, cyan) bound to two Fis1 cytoplasmic domains (TPR, orange; M, brown) anchored in the outer mitochondrial membrane (gray). The model was generated from crystal structures of the Fis1 cytoplasmic domain in complex with Mdv1 NTE-CC (Protein Data Bank, PDB [11–14] accession no. 3UUX). The carboxy-terminal β-propeller domain of Mdv1 is shown as a homology model based on the known structure of the Cdc4 WD40 repeat (PDB 1NEX). Loops of variable length and unknown structure connect the β-propeller domains to the Mdv1-CC dimer. Red indicates residues important for mitochondrial dynamin recruitment. See also [79,80,82].

Drp1–MiD49 heteropolymer would be narrow enough to drive fission of a mitochondrial compartment *in vivo*. Thus, some fission adaptors may co-assemble with, and change the physical properties of, mitochondrial dynamin assemblies in ways that enhance the ability of the dynamin to constrict and sever membranes.

The Role of Insert B in Mitochondrial Dynamin Membrane Recruitment

Understanding how mitochondrial dynamins recognize and bind their adaptors is an important issue. Two recent studies indicate that InsB domains are critical for this process [82,83]. This function makes sense, since InsB domains are predicted to reside at the base of the DRP oligomer, closest to the membrane. However, as described below, the mode of InsB function may vary in different organisms and depending upon the target adaptor.

A genetic screen identified a fungal-specific motif in yeast Dnm1 InsB essential for Dnm1 binding to the Mdv1 adaptor [82]. Mutations in this motif dramatically reduce Dnm1–Mdv1 interaction, abolish Dnm1 membrane recruitment and block mitochondrial fission. Suppressor mutations that rescue defects caused by InsB mutations cluster in the Mdv1 β-propeller, and localize to both surfaces of the modeled structure (Figure 3B, red). This observation is consistent with the finding that the Mdv1 β-propeller is sufficient for direct interaction with Dnm1 [82]. In cells expressing the Dnm1 InsB mutant and Mdv1 suppressor proteins, Dnm1 is once again recruited to the mitochondrial surface with a concomitant rescue of mitochondrial fission defects. Interestingly, the Mdv1 suppressor proteins interact better than wild-type Mdv1 with Dnm1, suggesting that the suppressor mutations create additional contacts with Dnm1. The residues identified in this suppressor screen must be in close proximity to InsB in order to make such contacts. Thus, they likely define potential binding interfaces on the Mdv1 β-propeller for InsB. These combined data strongly suggest that InsB functions as an adaptor-binding interface for Dnm1.

In mammals, InsB appears to negatively regulate interactions between Drp1 and the mitochondrial Mff adaptor protein. Drp1 has an alternatively spliced InsB domain that contains a number of sites for post-translational modification [84–86]. Using a combination of deletion and linker substitution mutagenesis, Strack and colleagues [83] showed that the Drp1 InsB domain is dispensable for Mff-mediated mitochondrial recruitment and mitochondrial fission. Moreover, complete deletion of the Drp1 InsB domain causes constitutive mitochondrial localization and excessive mitochondrial fission, suggesting that this domain normally has an autoinhibitory function. Although the location of InsB in the Drp1 structure is not known, it is proposed to interact with the stalk domain near interface 3. A specific residue in the Drp1 stalk near this site is required for Mff binding [83]. Thus, one interpretation of these results is that the Drp1 InsB domain functions to block a site on the Drp1 stalk where Mff binds.

Further studies, including structures, are needed to determine whether InsB domains of yeast and mammalian mitochondrial dynamins bind directly to adaptor proteins, or

mask adaptor interaction sites on the dynamin molecule. Generating such data will be challenging, since prediction algorithms indicate that most InsB domains in DRPs are disordered. This is probably why the Dnm1 InsB domain is missing in the Dnm1 cryo-EM structure [65]. It is possible that DRP InsB domains will adopt more stable conformations when bound to fission adaptors, which would facilitate structural analysis. In the case of Drp1, it will also be necessary to identify the cellular mechanisms that counteract the autoinhibitory function of InsB. Understanding how different spliced isoforms and post-translational modifications (phosphorylation, sumoylation and S-nitrosylation) of Drp1 impact InsB–adaptor interactions is another area ripe for study. These isoforms and post-translational modifications and their known physiological roles have been reviewed elsewhere [84–86].

Co-Evolution of InsB Domains and Fission Adaptors

Although mitochondrial fission is conserved in eukaryotes, the physiological roles of this process have evolved and become more complex in multicellular organisms. We argue that this increased complexity has been accommodated by an expansion of fission adaptor proteins. As noted above, most of the mitochondrial fission adaptors in different organisms are not related by primary amino acid sequence or predicted secondary structure. Moreover, the adaptors seem to have appeared at different points during evolution. While the β-propeller-containing adaptors are conserved in fungi and algae [87,88], Mff is conserved in metazoans [24] and MiDs are vertebrate-specific adaptors [25]. Further investigation should reveal whether these diverse adaptors utilize similar or different strategies to bind and recruit their respective mitochondrial dynamins to the membrane. We propose that sequence variation in the InsB domains of mitochondrial dynamins was one strategy used to accommodate this adaptor diversity. In support of this idea, alignment of mitochondrial DRPs from different organisms reveals little sequence conservation in the InsB domain as one moves from fungi to vertebrates. For example, the motif in the Dnm1 InsB domain that is required for Mdv1 interaction is only conserved among the Dnm1 fungal homologs. Mammalian Drp1 harbors an entirely different InsB sequence. In some cases, the InsB domain may facilitate recognition and/or binding to a specific fission adaptor. This is clearly the case for the motif in Dnm1 that mediates Mdv1 interaction [82]. Alternatively, InsB domains could negatively regulate adaptor binding as described for mammalian Drp1 and Mff [83]. Additional functions for InsB will likely be identified as investigations proceed.

Concluding Remarks

One theme of this review is the manner in which strategies for classical dynamin assembly and function have been adapted by the related GTPases that divide mitochondria. Indeed, much of what we currently understand about Dnm1/Drp1 activity comes from experiments modeled on studies pioneered in the dynamin field. The introduction of a diverse group of essential adaptor proteins to the system signals a departure from the dynamin model. Understanding how these different adaptors interact with mitochondrial dynamins to regulate their recruitment, assembly and mechanism of action will keep researchers busy for the next few years. Developing robust systems to dissect the physiological functions of different adaptors will also be essential for future analyses.

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